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Absorption, Translocation and metabolism of 1,3-dichloropropene in selected plants

David L. Berry

ABSORPTION, TRANSLOCATION AND METABOLISM OF 1,3-DICHLOROPROPENE IN SELECTED PLANTS

by

David L. Berry

A dissertation submitted in partial fulfillment
of the requirements for the degree
of
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in
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Approved:

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David L. Berry

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

ABSTRACT

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1,3-dichloropropene in selected plants

by

David L. Berry, Doctor of Philosophy

Utah State University, 1973

Major Professor: Dr. D.K. Salunkhe

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Interdepartmental Curriculum: Toxicology

The absorption, translocation and metabolism of 1,3-dichloropropene (a soil fumigant) in bush beans, tomato and carrot was studied under growth chamber and greenhouse conditions using solution culture, vermiculite and sand. Absorption was monitored using gas chromatographic analysis and isotope techniques. Plants were shown to absorb a maximum amount of dichloropropene by 24 to 48 hr from solution culture and vermiculite. The plant absorbed and translocated 1,3-dichloropropene-^{14}C-U readily to aerial parts of the plant.

Bush beans, tomatoes and carrots absorbed and translocated 3-chloroallyl alcohol-^{14}C-U from solution culture and vermiculite. Levels of 3-chloroallyl alcohol reached maximum at 24 to 48 hr after inoculation. The gas chromatographic analysis of plant materials showed that 1,3-dichloropropene and 3-chloroallyl alcohol were

rapidly metabolized by the plant. The three plants metabolize 1,3-dichloropropene to 3-chloroallyl alcohol, part of which is converted to 3-chloroacrylic acid and 3-chloro-1-propanol. The metabolite identities were confirmed by co-chromatography with standard compounds and by mass spectral analysis. The sequence from this point (3-chloroacrylic of 3-chloro-1-propanol) is not known but coupled with the evidence from metabolite studies, it is apparent that a central metabolite (acetate pathway is indicated) has to be an intermediate in dichloropropene metabolism as label is located in glucose, TCA acids, amino acids, lipids and other normal plant products.

The dichloropropenes are rapidly absorbed, translocated and metabolized by the plant. No parent dichloropropene was found in the plant after 72 hr incubation period and 3-chloroallyl alcohol was not detected after 96 hr in the plant. The data indicates that the dichloropropenes and 3-chloroallyl alcohols are not potential residue problems and that envirnomental concern about the ultimate fate of these compounds should be minimal.

(pages)

INTRODUCTION

The agricultural industry spends in excess of $ 1.7 billion annually for agricultural chemicals to control and eliminate various pests affecting agricultural production. The soil fumigants comprise in excess of $ 15 million of the total sales and their use is increasing. The majority of the fumigants are used to combat plant parasitic nematodes such as root knot nematodes, golden nematodes and lesion nematodes. The effective fumigants in use are small molecular weight halogenated hydrocarbons with a high vapor pressure so that they will volatilize and diffuse through the soil profile.

Many of the agricultural chemicals used today are applied so that they come in direct contact with the soil and are intimately associated with the plant root system. Organochlorine insecticides such as DDT and dieldrin have been shown to be absorbed and translocated into ediable portions of carrots,peas and other crops. Organophosphate and carbamate insecticides are absorbed and metabolized by plants and soil microbes. The soil fumigants ethylene dibromide and methyl bromide are absorbed and metabolized by the plant but information on plant metabolism of dichloropropene containing fumigants is nonexistant.

Telone^{R} (a mixture of cis- and trans-1,3-dichloropropene, 1,2-dichloropropane and epichlorohydrin) is an extensively used soil fumigant for the control of nematodes. The major constituent of this mixture is 1,3-dichloropropene (1,3-DCPE) and it is usually injected into the moist soil at a depth of 8 to 20 inches approx-

imately two weeks before planting. Nematocidal levels of 1,3-DCPE have been extensively studied by Castro at U.C. Riverside. They have found residues of the parent compound in the soil as well as some of the metabolites of the dichloropropenes. They have demonstrated that Pseudonomas sp metabolize 1,3-DCPE to CO_2 and water via 3-chloroallyl alcohol, 3-chloroacrylic acid and malonic semi-aldehyde. Their work and information from other laboratories indicate that 1,3-DCPE does not remain in the soil long and that the only detectable chemical in the soil after a 9 month growing season is 3-chloroallyl alcohol.

Wu et al. has shown that carrots, peas and corn grown on soils treated with Telone^R and Nemagon^R (1,2-dibromo-3-chloro-propane) produce more soluble sugars and carotenes and have a lower respiration rate. Karasz and Gantenbein working with potatoes could find no detectable 1,3-DCPE in the plant after a growing season. Tams working with pineapples found no detectable 1,3-DCPE after a growing season. Previous studies, however, have centered only with long term residues and effects of the dichloro-propenes.

This study is directed toward the short term effects and residues of the dichloropropenes. Specifically, is the plant absorbing 1,3-DCPE and what is its fate in the plant. If it is being absorbed, how does the plant translocate and metabolize 1,3-DCPE so that residues are not found in the plant after a growing season? What are the metabolites of 1,3-DCPE, does it form conjugates and what is the turnover rate of 1,3-DCPE in the plant system?

REVIEW OF LITERATURE

The dichloropropene soil fumigants are utilized in agricultural production for the control of root knot nematodes, lesion nematodes, cyst formers and golden nematodes. They are applied previous to planting by injection into the soil and allowed to diffuse within the soil profile. Telone® (a mixture of cis- and trans-1,3-dichloropropene, epichlorohydrin and 1,2-dichloropropane) (Dow Chemical Co., Midland, Mich.) and other dichloropropene containing products have been in production since the early 1950's and continue to be used extensively because of their toxicity to plant parasites, their behavior in soils and their minimal residues.

The chemical and physical characteristics of the dichloropropenes as well as the acute mammalian toxicity and other toxicological considerations were reported by Fletcher (1956). Utaka (1963) reported refined boiling points of the cis- and trans- isomers and spectral data on the dichloropropenes.

The vapor pressure of the dichloropropenes provides the physical principles for the diffusion of the dichloropropenes in the soil. Goring (1962) has defined some of the soil variables which influence the diffusion of the dichloropropenes in the soil system. Jurinak (1957, 1960) has shown that two related fumigants, ethylene dibromide and 1,2-dibromo-3-chloropropane, react with the soil surface and that organic matter and moisture affect the reaction. Hannon et al. (1963) and Williams (1968) have shown that soil type and moisture affect the recovery of the dichloropropenes from soil extracts. Leistra (1970) has shown

that the trans- isomer of 1,3-dichloropropene is more tightly bound to the soil than the cis- isomer. Eloquent information on the diffusion of the dichloropropenes in organic soils has been provided by Leistra (1971) and information regarding sandy loam soils by McKenry (1972). Diffusion patterns of 1,2-dibromo-3-chloropropane, a similar fumigant, have been reported by Walla (1972).

The biological activity of the dichloropropenes appears related to the diffusion of the compounds and the allylic structure as reported by Moje (1959). The effects of saturated dichloropropanes on the activity of the unsaturated dichloropropenes have been shown to be slight (Moje (1963) and Youngson and Goring (1970)). The effects of water and other environmental factors on the biological activity of the dichloropropenes have been elucidated by Marks et al. (1968).

The metabolism of the organo-halide compounds in the soil system is both biological and non-biological in character. Castro and Bartnicki (1965) have shown that a soil Pseudomonas sp. can convert 3-bromo-1-propanol to 3-bromopropionic acid and eventually CO_2. Castro and Belser (1968) have proposed a series of reductive dehalogenations of ethylene dibromide, 1,2-dibromo-3-chloropropane and 2,3-dibromobutane. Castro and Bartnicki (1968) have isolated a Flavobacterium sp. from soil that is capable of biodehalogenation via epoxide opening and transhalogenation. Castro and Belser (1966) demonstrated hydrolysis of cis- and trans-1,3-dichloropropene to their corresponding 3-chloroallyl

alcohols and stated that this was a hydrolysis reaction as well as a biological process. Belser and Castro (1971) further isolated a soil Pseudomonas sp. capable of metabolizing 3-chloroallyl alcohol to 3-chloroacrylic acid and eventually CO_2. Ruddick (1972) has isolated and characterized the metabolism of 1,2-propanediol by soil Pseudomonas sp., Flavobacterium sp. and a Penicillium sp.

Plants grown on soils fumigated with the organohalide compounds show varying responses from increased vigor and phytotoxicity with the dichloropropenes to increased vigor and non-phytotoxicity with 1,2-dibromo-3-chloropropane. Tams (1945) indicated that D-D^R (50% 1,2-dichloropropane and 50% 1,3-dichloropropene) (Shell Chemical Co., San Rafael, Cal.) increased soil nitrification and improved the vigor of pineapple. McCantz et al. (1959) reported that tobacco grown on D-D^R treated soils out yielded non-treated soils. Wolcott et al. (1960) indicated that Telone^R inhibited nitrification and that celery grown on treated soils did not produce well. Altman and Tsue (1965) demonstrated that soil fumigation with D-D^R effectively increased the germination of sugar beets and that growth of the greens seemed stimulated. Whitehead et al. (1970 a) revealed control of migratory root-parasite nematodes and increased yields in sugar beets following fumigation with D-D^R. Whitehead et al. (1970 b) reported increased growth in barley and increased sugar content in sugar beets grown on D-D^R and Telone^R treated soils. Emerson et al. (1969) reported that quality and nutritive value of selected vegetables was enhanced when grown on soils treated with

Telone[R] and other soil fumigants. Wu et al. (1970) and Salunkhe et al (1971) indicated that respiration rates were decreased and carotenoid content increased in carrots and corn grown of Telone[R] and Nemagon[R] (1,2-dibromo-3-chloropropane) (Shell Chemical Co., San Rafael, Cal.). In addition, Wu and Salunkhe (1971) reported that the ultrastructure of the chromoplast was altered in carrots grown on dichloropropene treated soils.

The uptake and metabolism of organic pesticides by the plant has been recently reviewed by Casida and Lykken (1969). They indicated that the plant can absorb organochlorine and organophosphate compounds as well as other commonly used pesticide compounds. They reported that methyl bromide can be transformed by the plant to inorganic bromine and methanol. They stressed, however, that little is known about the metabolic transformation of zenobiotics in the plant. Young (1971) reported that pineapples grown on D-D[R] and Telone[R] treated soils contained no residue traces of the dichloropropenes. Karasz and Gantenbein (1971) reported similar findings with potatoes grown on D-D[R] or Vorlex[R] (2,3-methyl isothiocyanate) (Morton Chemical Co., Woodstock, Ill.) treated soils.

Thomason et al. (1971) stated that very little is known about the soil fumigants after nematode control. He reviewed the fate of fumigants once they have entered the soil in physical and microbiological terms. The biological transformations in the soil are well defined but little is known as to the metabolism of the fumigants in the nematode and in other systems such as the

plant. In fact, the specific mode of toxic action is not known for these compounds and they have been used for almost 20 years. Thus, the total mass budget of the dichloropropene soil fumigants is not known and is incomplete. It lacks the metabolism of the dichloropropenes in nematodes and more importantly, in the plant.

EXPERIMENTAL

Preparation of the plant materials

Carrots (Daucus carota, cv. Red Chatney) were grown in a glass house in vermiculite or sand. Bush beans (Phaseolus vulgaris L., cv. Tender Green) were grown in vermiculite in a growth chamber (Sherer-Gillett, Model No. CEL 37-14) at 3500 ft-c, 26 C day, 21 C night and 16 hr day. Tomatoes (Lycopersicon esculentum L., cv. VF-7) were grown under the same conditions as the bush beans. The beans were planted and watered with half strength Hoagland's solution (Arnon and Hoagland, 1940) until they reached the third trifoliate stage. Subsequently, the plants were thinned and selected for uniformity. Tomato plants were grown and watered with Hoagland's solution until they reached a height of 7 to 8 in. Carrots were planted in sand and vermiculite and watered with Hoagland's solution. The carrots grown in sand were grown to maturity while the plants grown in vermiculite were raised for 10 to 12 weeks.

Long term metabolic studies employing 1,3-dichloropropene-^{14}C-U (1,3-DCPE-^{14}C-U) and 3-chloroallyl alcohol-^{14}C-U (3-CAA-^{14}C-U) were undertaken using carrots grown in sand. The isotopes were injected into the root zone after the plants had become established and the plants were harvested at the end of the growing season and frozen. The sand was frozen and sampled for the presence of 1,3-DCPE-^{14}C-U and 3-CAA-^{14}C-U.

Chemicals

Organic solvents were supplied by J.T. Baker Chemical Co., Philadelphia, Penn. and Mallinckrodt Chemical Works, St. Louis, Mo. Diethyl ether, 2,2,4-trimethyl pentane and hexane were reagent grade and distilled over sodium wire in a 6 ft Vigreaux column. Amino acids, sugars and organic acids were purchased from Nutritional Biochemicals, Cleveland, Ohio and Sigma Chemical Co., St. Louis, Mo. Trifluoroacetic anhydride was purchased from Pierce Chemical Co., Rockford, Ill. Organic acids were derivatized for GLC analysis using Sylon BTZ, a product of Supelco, Inc., Bellefonte, Penn. Liquid scintillation (LSC) was performed using a xylene based counting cocktail Aquasol^{R} which can accommodate up to 20% water in the counting system and is a product of New England Nuclear Corp., Boston, Mass.

The Dow Chemical Company of Midland, Mich. supplied standards of cis- and trans-1,3-dichloropropene (c-1,3-DCPE and t-1,3-DCPE) and cis- and trans-3-chloroallyl alcohol (c-3-CAA and t-3-CAA). The purity of the standards was determined by GLC and reported as follows: cis-1,3-dichloropropene 98.2% and 1.2% trans-; trans-1,3-dichloropropene 07.2% and 2.1% cis-; cis-3-chloroallyl alcohol 96.2% and 1.6% trans-; and trans-3-chloroallyl alcohol 97.4% and 1.8% cis-. Samples of 1,3-dichloropropene-^{14}C-U (60% cis and 40% trans) and 3-chloroallyl alcohol-^{14}C-U (60% cis and 40% trans) were synthesized and supplied by The Dow Chemical Co. Samples of trans-3-chloroacrylic acid (t-3-CAcryl), 3-chloropropionic acid,

3-chloro-1-propanol and n-octanol were purchased from Aldrich Chemical Co., Milwaukee, Wis.

Metabolic investigations

Administration of 1,3-dichloropropene-^{14}C-U. Beans and tomatoes were placed in a half strength Hoagland's solution. The labelled dichloropropene was given to the plant emuslified with 2% Triton B-1956 (Rohm and Haas, Philadelphia, Penn.) and allowed to incubate for 1 to 24 hr under a 16 hr regime. The plants were harvested, frozen and stored at -20 C until extraction. The nutrient solution was salted and extracted with hexane to determine remaining isotope in the nutrient solution. Carrots were exposed to the labelled dichloropropenes in moist sand or vermiculite for 1 to 24 hr.

Administration of 3-chloroallyl alcohol-^{14}C-U. Beans and tomatoes were exposed to labelled 3-chloroallyl alcohol in a Hoagland's nutrient culture for 1 to 24 hrs. The plants were harvested, frozen and stored at -20 C until extraction. Carrots were exposed to the isotope in moist sand or vermiculite for a period of 1 to 24 hrs. The isotope solution was emulsified with 2% Triton BO1956. The nutrient solution was salted and extracted with hexane to determine the amount of unincorporated isotope in solution.

Administration of non-labelled compounds. Beans and tomatoes were grown in vermiculite until they reached the desired stage of maturity. Samples of cis- and trans-1,3-DCPE (50:50;v/v) were emulsified with Triton B-1956 and injected directly into the

root zone. The plants were allowed from 0.5 to 120 hrs contact with the compounds and subsequently frozen and stored at -20 C until extraction. The same procedure was followed for the 3-CAA using beans and tomatoes. Carrots were grown in vermiculite in the glass house until the desired stage of maturity was reached. They were exposed to the 1,3-DCPE and 3-CAA for 1.0 to 120 hrs and frozen until extraction.

Extraction of metabolites

Labelled compounds. Plant samples were homogenized and extracted according to the scheme of Foy (1969) with modifications. Plants were forzen, powdered, homogenized and extracted with 80% ethanol, neutral ether (pH 7), basic ether (pH 13) or acidic ether (pH 1).

The neutral ether extract was concentrated and washed with 10% aqueous Na_2CO_3 to remove organic acids, transferred into petroleum ether and washed with 80% ethanol to separate xanthophylls and lipids from the chlorophylls and carotenes. The resulting extracts were separated using thin layer chromatography (TLC) (20 x 20 cm plates) coated with 0.5 mm Silica Gel G (Brinkmann Istruments, Inc., Westbury, New York) developed in benzene:petroleum ether (60:40;v/v) for the pigments or petroleum ether:benzene:water (100:20:7; v/v) for the xanthophylls. The labelled compounds were scraped from the plates into vials and counted in a liquid scintillation counter (LSC). The compounds were identified using co chromatography with known standard compounds.

The acidic ether extract was concentrated and washed with 10%

Na_2CO_3 to separate the anionic acids and pigments. The ether fraction was spotted on a TLC plate and developed as described above. The aqueous phase was passed through an anionic exchange resin (Dowex 1x-8, formate form) , eluted off the resin with 1 N NH_4OH, pH 10-11, brought to pH 1.5 with 6 N HCl, extracted with ether and spotted on a paper chromatogram (PC) or counted directly.

The basic ether extract contained some freely saponifiable lipids, cationic acids and pigments. The pigments and lipids were separated from the acids by washing with 10% aqueous Na_2CO_3. The ether extract was spotted on TLC plates coated with Silica Gel G and developed in either petroleum ether:benzene (60:40; v/v) or n-butanol:acetic acid:water (12:3:5;v/v).

The 80% ethanol extracts were filtered and concentrated in vacuo or in a vigreaux column. The concentrate was passed first through an anionic exchange resin (Dowex 1X-8, formate form, 2.54 x 20 cm) on which the metabolic acids and some phosphorylated compounds were absorbed. The effluent concentrate was passed through a cation exchange resin (Dowex 50X-8, H^+ form, 2.54 x 20 cm) which absorbed principally cationic amino acids. The nonabsorbed concentrate contained neutral sugars and pigments.

The metabolic acids and phosphorylated compounds were eluted off the resin by washing with 1 N NH_4OH, pH 10-11. The resin was removed from the slurry by filtration and the filtrate was lowered to ph 1.5 by addition of 6 N HCl. The filtrate was extracted with ether and spotted on Whatman No. 1 paper (50 x 50 cm)

and developed two-dimensionally using the method of Kliewer (1966) and modified solvent systems of n-butanol:propionic acid:water (12:3:5;v/v) and saturated phenol:ammonia:water (80:5:15;v/v). Spots were identified by method of Paskova and Munk (1960) using bromocresol green, pH 4.5. Keto acids were determined as their 2,4-dinitrophenyl hydrazone derivatives according to Isherwood and Niavis (1956), Meister and Abendschein (1956) and Kliewer (1966).

The cationic amino acids were eluted from the cationic resin by washing with 1 N H_2SO_4, pH 1 and filtering the slurry. The filtrate was spotted on Whatman No. 1 paper (50 x 50 cm) and developed as described by Kliewer (1966).

The neutral sugars and pigments were spotted on Whatman No. 1 paper (50 x 50 cm) and developed one-dimensionally using n-butanol:ethanol:water (40:11:19;v/v) or ethyl acetate:acetic acid:formic acid:water (18:3:1:4;v/v) as described by Kliewer (1966). The sugars were located by spraying with p-anisidine reagent as described by Pridham (1966) and identified by co-chromatography with known standards.

Quantification of the label in all cases was carried out by cutting the spots from the PC or scraping the spots from TLC into scintillation vials and counting the activity using LSC.

Residue studies for 1,3-dichloropropene, 3-chloroallyl alcohol and immediate metabolites

Samples of frozen plants were powdered (beans and tomatoes)

(sliced in the case of carrots), weighed to 1 gm and homogenized twice in a high speed blender with 50 ml 2,2,4-trimethyl pentane (alternatively 50 ml hexane) and 20 ml methanol. The homogenate was filtered through polyester cloth and 200 ml of water saturated with $(NH_4)_2SO_4$ were added and the mixture shaken. The combined extract was centrifuged at 5000 x g for 5 min and the organic phase drawn off. The organic fraction was passed through a Florosil column as described by Morley (1966) and Wheeler and Frear (1966). The resultant organic phase was used for GLC analysis.

GLC analysis

Residue analysis of 1,3-dichloropropene, 3-chloroallyl alcohol and immediate metabolites was performed on a Packard Model 804 gas chromatograph equipped with a nickel-63 electron capture detector. A circular glass column 8 ft by 4 mm id was packed with 10% Carbowax 20 M on Chromosorb W (HP) (Pierce Chemical Co., Rockford, Ill.) and was used for separation of the dichloropropenes and metabolites. The column was packed, pre-conditioned at 180 C for 96 hr and operated isothermally at 120 C. The operating parameters were as follows: injection temperature, 180 C; column temperature, 120 C; detector temperature, 250 C and carrier gas flow rate, 50 mls/min. This method for dichloropropene analysis was that of Ramsey (1972).

The 3-chloroallyl alcohol did not give a sharp peak on the GLC so it was necessary to derivatize the alcohol first with trifluoroacetic anhydride (TFA) and wash with 1% $NaHCO_3$. The 3-CAA-TFA was injected into the carbowax column operated at 90 C and a flow rate

of 40 ml/min. The methods of Cole and Crank (1971) and Irvine and Saxby (1969) were used to make TFA derivatives of 3-CAA and 3-chloro-1-propanol.

The 3-chloroacrylic acid and 3-chloropropionic acids were chromatographed on a carbowax column operated at 90 C. The two acids were derivatized to their trimethylsilyl esters by addition of Sylon BTZ^{R} to a concentrated extract and allowed to react at room temperature for 1 hr. The resulting derivative was subjected to GLC.

Standards were prepared every-other-day, column profiles and standard curves were determined daily and compounds were identified by comparison of retention times with known standard compounds.

Radioautography

Bean plants were exposed to 1,3-DCPE-^{14}C-U in nutrient culture for 1 to 24 hr. The plants were removed, blotted dry, mounted on fiber board, desiccant added and wrapped with saran wrap. The mounted plant was placed in a Kodak X-ray envelope containing Kodak No-Screen X-ray film and exposed for 21 days. The X-ray film was developed and prints made from the resulting autoradiograms.

Octanol/water partition coefficient

The octanol/water partition coefficient for 1,3-DCPE and 3-CAA was determined since the volatility prevented determination of

p-values by TLC. Samples of 1,3-DCPE-^{14}C-U and 3-CAA-^{14}C-U were placed in equal volumes of n-octanol and water, shaken for 5 min and allowed to equilibrate fo 1 hr and the phases separated. Aliquots of the organic and aqueous phase were placed in scintillation vials and counted using LSC. The p-value reported is:

p-value = [Conc. organic phase]/[Conc. aqueous phase]

GC-MS spectra analysis

Samples of dichloropropenes and selected dichloropropene metabolites were concentrated in a vigreaux column and subjected to GC-MS analysis. A Varian Aerograph Model 205 GC (a 6 ft x 2mm id glass column packed with 1% OV-1 on Chromosorb W (HP) operated at 50 C and a flow rate of 10 ml/min) was connected to a Finnegan Mass Analyzer and selected masses were analyzed. These analyses were used to confirm GLC data and were provided by Dr. D.G. Crosby, Dept. of Environmental Toxicology, University of California at

Statistical analysis

All experiments were planned in a randomized block design with a minimum of 4 replications. Standard curves were plotted and subjected to regression analysis. Means, standard deviations and analysis of variance (where pertinant) were performed according to the procedures described by Little and Hills (1972).

RESULTS

Absorption of 1,3-dichloropropene

Bush beans were exposed to 1,3-dichloropropene-^{14}C-U in solution culture for 24 hr as shown in Figure 1. The plants were removed from the nutrient culture and autoradiograms were made of the whole plant. The autoradiograms showed that the label was distributed throughout the plant at the end of a 24hr period (Figure 2).

The normal route of uptake of the dichloropropenes from the soil is through the root system of the plant. A comparison of uptake by the root, by the excised shoot and by topical contact with the leaf showed that the plant absorbs the dichloropropenes from all three routes. The plant absorbs the dichloropropenes most effectively through topical application through the leaf followed by excised shoot and through the root system. The plant translocates the material throughout the whole plant when absorbed through the root system while the dichloropropenes are only slightly translocated when absorbed through the leaf (Table 1).

The absorption of the dichloropropenes through the roots from a nutrient culture solution appears to be time dependent. The uptake of the labelled material begins slowly and by 24 hr has reached maximum or plateau level (Figure 3). The bean plant was shown to absorb from 55 to 75% of the label administered to the plant over a 24 hr period of exposure. The label was first noted

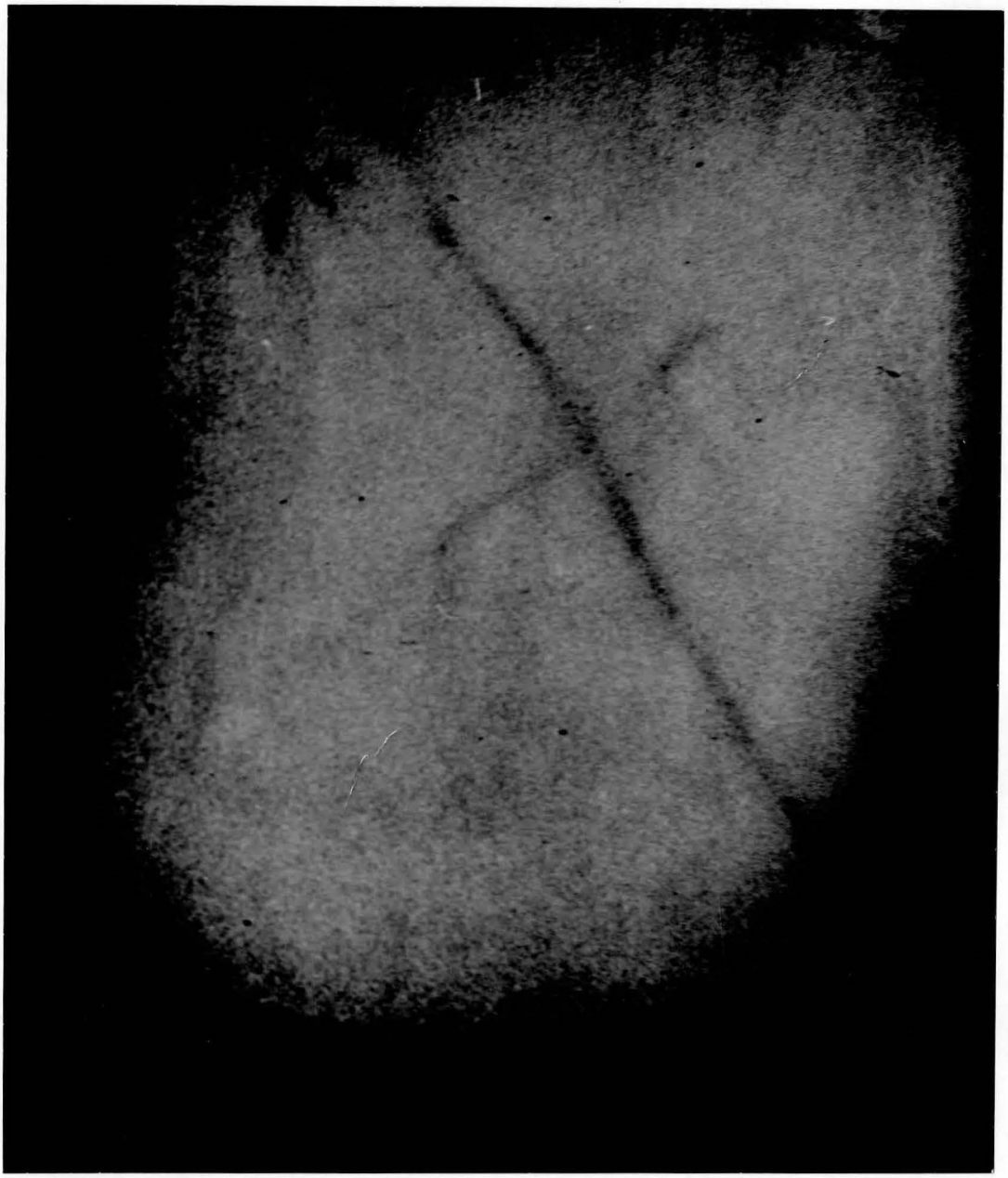

Figure 1. Autoradiogram of bush bean after 24 hr exposure to 1,3-dichloropropene-^{14}C-U

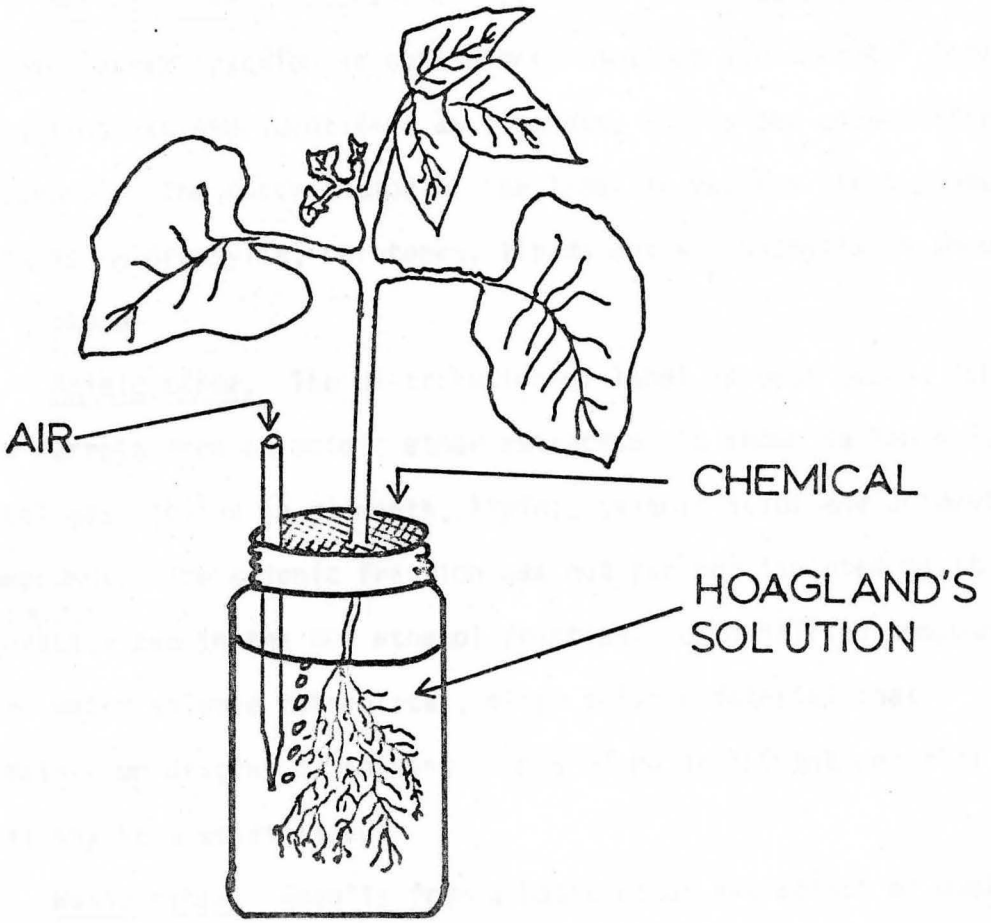

Figure 2. Plant and solution culture

in the root zone at 4 hr and gradually increases in the stem and leaf. Maximum absorption occurs after 24 hr incubation with the majority of the label located in the root and stem (Table 2).

Distribution of the label from 1,3-dichloropropene-^{14}C-U

Neutral ether. Label from 1,3-DCPE-^{14}C-U appeared in the neutral ether fraction in bush beans, tomatoes and carrot. Compounds were isolated and identified as pigments, lipids and unidentified compounds. The distribution of the label in various plant products such as chlorophylls, carotenes, lipids and xanthophylls is shown in Table 3.

Acidic ether. The distribution of label in bush beans, tomatoes and carrots from an acidic ether extraction is shown in Table 4. Label was located in pigments, lipids, anionic acids and unidentified compounds. The anionic fraction was not further isolated as it was characterized in the 80% ethanol fraction. Unidentified compounds were water soluble metabolites, ether soluble material that remained on origins or solvent fronts of PC or TLC and material that may have volatilized.

Basic ether. Results from a basic ether extraction of bush beans, tomatoes and carrots are given in Table 5. The carotenes contained the majority of the label in the pigment fraction. Lipid and easily saponifiable lipids contained label. Cationic acids contained most of the label in a basic ether extract followed by unidentified compounds.

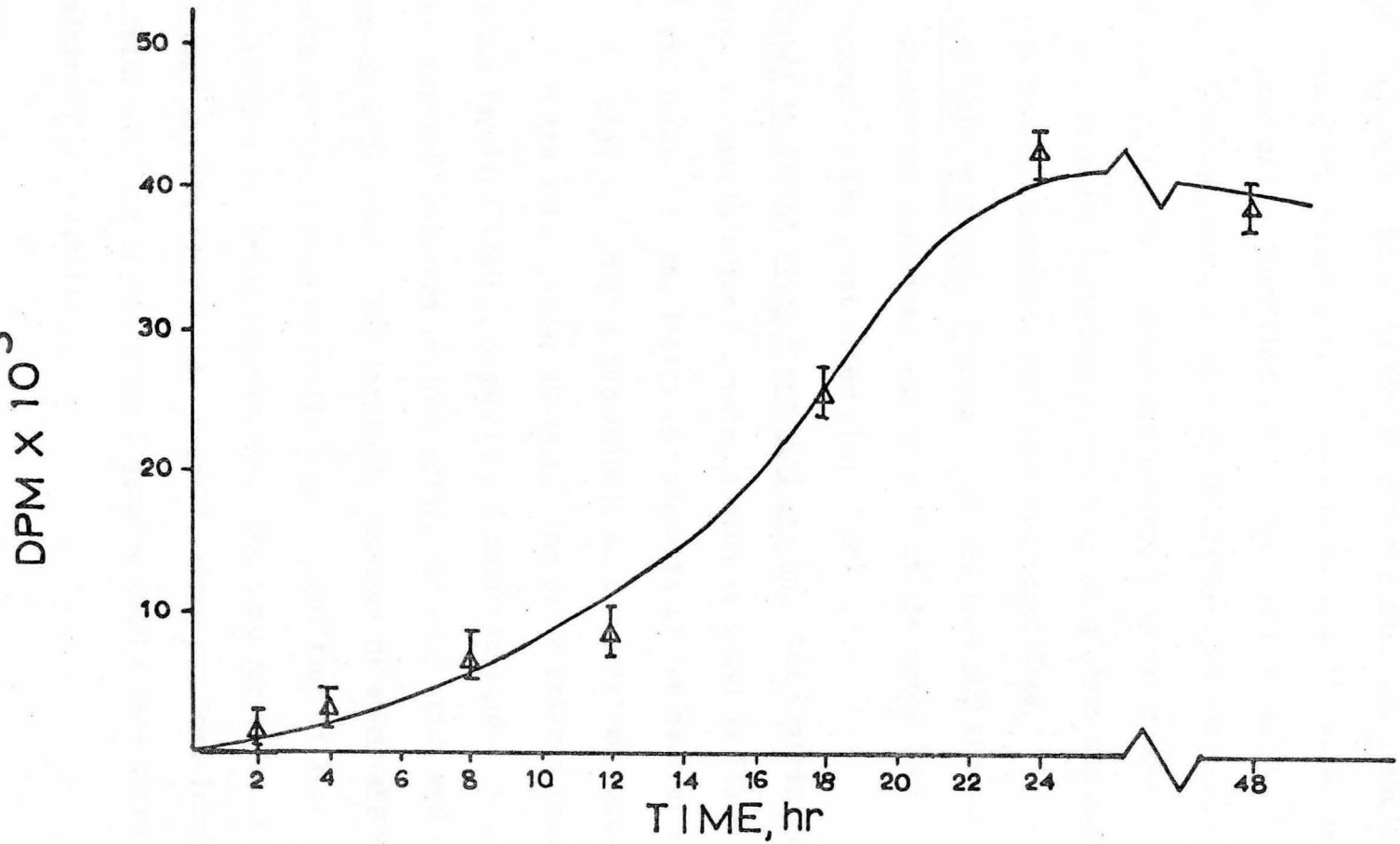

Figure 3. Absorption of 1, 3-dichloropropene-^{14}C-U in bush beans

80% Ethanol. Results from an 80% ethanol extraction of bush beans, tomatoes and carrots are sumarized in Table 6. The fractions were separated into anionic acids, cationic acids and neutral compounds. Label in the anionic fraction was associated with citric acid, succinic acid, fumaric acid, malic acid, α-keto-glutaric acid and unidentified acids. The cationic acids fraction contained labelled amino acids such as alanine, aspartic acid, glutamic acid, glycine, serine and unidentified cationic acids. The neutral fraction contained sugars such as glucose and sucrose and other neutral compounds that were not identified.

Insoluble residues. Fibrous residues from 80% ethanol and ether extractions contained less that 1% of the total label administered to the plant after 24 hr (Table 7).

Label in carrot after 1 growning season. The distribution of label in carrots after 1 growing season is shown in Table 8. Label was located in the lipids and pigments of the neutral ether fraction. Lipids, pigments and anionic acids contained labelled material in the acidic ether extract. The basic ether extract contained labelled lipids, pigments and cationic acids. The 80% ethanol extract contained anionic acids, cationic acids and neutral coumpounds with label. The insoluble residues of ether and ethanol extracts contained proportionally higher quantities of label than the short term labelling experiments. The sand contained residual label that was not identified. Overall, there was less label associated with the carrot after 1 growing season than there was after 24 hr incubation.

Table 1. Incorporation of 1,3-dichloropropene-^{14}C-U into bush bean as a function of route of administration and translocation of 1,3-dichloropropene-^{14}C-U within the plant.

Source	Counts, dpm	% Incorporated	% of Total Incorporated
Initial added[a] 61,500			
Whole plant, 24 hr			
Leaf	11,785	19	27
Stem	23,622	38	54
Root	8,973	15	19
Total	44,380	72	100
Excised plant, 24 hr			
Leaf	11,115	18	28
Stem	28,490	47	72
Total	39,605	65	100
Topical plant, 24 hr			
Leaf	39,640	64	83
Stem	7,136	12	15
Root	972	2	2
Total	47,748	78	100

[a] Initial amount of isotope added to the plant in Hoagland's solution.

Table 2. Distribution of label from 1,3-dichloropropene-^{14}C-U in bush beans with respect to time.

Source	Counts, dpm	% Total
Initial added[a]	61,500	
Whole plant, 4 hr		
Leaf	650	
Stem	720	
Root	1,287	
Total	2,657	4
Whole plant, 8 hr		
Leaf	3,326	
Stem	1,148	
Root	1,065	
Total	5,539	9
Whole plant, 24 hr		
Leaf	1,874	
Stem	26,748	
Root	13,264	
Total	41,886	68
Whole plant, 48 hr		
Leaf	1,246	
Stem	25,982	
Root	11,497	
Total	38,725	63

[a] Initial added to the plant in a Hoagland's solution.

Table 3. Distribution of label from 1,3-dichloropropene-^{14}C-U in neutral ether (pH 7) extract from bush bean, tomato and carrot after 24 hr.

Fraction	Bean[b] counts, dpm	Tomato[b] counts, dpm	Carrot[b] counts, dpm
Initial added[a]	55,480		
Chlorophyll	10	7	--
Carotene	45	92	86
β-carotene	12	18	26
Other pigments	185	318	176
Lipids	165	143	240
Xanthophylls	10	46	22
Unidentified	1386	1064	1640

[a] Initial amount of label administered to the plant in a nutrient solution.

[b] Average values from a minimum of 4 replictions.

Table 4. Distribution of label from 1,3-dichloropropene-^{14}C-U in acidic ether (pH 1) extract from bush bean , tomato and carrot after 24 hr.

Fraction	Bean[b] counts, dpm	Tomato[b] counts, dpm	Carrot[b] counts, dpm
Initial added[a] 52,400			
Chlorophyll	26	14	11
Carotenes	286	524	178
Other pigments	146	182	112
Xanthophylls	124	46	--
Lipids	213	271	187
Anionic acids	4836	2765	1648
Unidentified	1642	1983	1214

[a] Initial amount of label administered to the plant in a nutrient solution.

[b] Average values from a minimum of 4 replications.

Table 5. Distribution of label from 1,3-dichloropropene-^{14}C-U in alkaline ether (pH 13) extract from bush bean, tomato and carrot after 24 hr.

Fraction	Bean[b] counts, dpm	Tomato[b] counts, dpm	Carrot[b] counts, dpm
Initial added[a] 49,100			
Chlorophyll	10	--	--
Carotenes	164	125	186
Other pigments	108	216	87
Xanthophylls	38	41	--
Lipids	58	47	112
Saponifiable lipids	62	71	246
Cationic acids	1864	1346	847
Unidentified	763	428	596

[a] Initial amount of label administered to the plant in a nutrient solution.

[b] Average values from a minimum of 4 replications.

Table 6. Distribution of label from 1,3-dichloropropene-^{14}C-U in 80% ethanol extract from bush bean, tomato and carrot after 24 hr.

Fraction	Bean[b] counts, dpm	Tomato[b] counts, dpm	Carrot[b] counts, dpm
Initial added[a] 16,400			
Anionic acids			
Citric	175	108	136
Succinic	87	165	114
Fumaric	64	96	83
Malic	73	76	64
∝-ketoglutaric	62	116	95
Unidentified	483	336	274
Cationic acids			
Alanine	92	84	74
Aspartic	65	136	62
Glutamic	59	48	41
Glycine	47	66	52
Serine	22	37	31
Unidentified	183	216	143
Neutral Compounds			
Glucose	562	492	381
Sucrose	1096	957	857
Unidentifed	573	635	721

[a] Initial added to plant for incubation.

[b] Average values from 4 replications.

Table 7. Distribution of label from 1,3-dichloropropene-^{14}C-U in insoluble residues of bush bean, tomato and carrot after 24 hr.

Fraction		Bean[b] counts, dpm	Tomato[b] counts, dpm	Carrot[b] counts, dpm
Initial added[a]	49,100			
Ether insoluble[c]		186	143	236
80% ethanol insoluble		265	391	197

[a] Initial added to plant nutrient solution.

[b] Average values from 4 replications.

[c] Label associated with insoluble material. No further attempt to identify was performed.

Table 8. Distribution of label from 1,3-dichloropropene-^{14}C-U in carrot after 1 growing season (6 months).

Fraction	Ether[c]			80% Ethanol
	Neutral counts, dpm[b]	Acidic counts, dpm[b]	Alkaline counts, dpm[b]	counts, dpm
Initial added[a]	61,500			
Chlorophyll	13	17	14	--
Carotenes	46	54	43	--
Other pigments	36	26	37	--
Lipids	37	58	40	--
Saponifiable Lipids	--	--	63	--
Anionic acids	--	142	--	--
Cationic acids	--	--	106	--
Unidentified	86	208	162	--
Citric acid	--	--	--	10
Succinic acid	--	--	--	--
Fumaric acid	--	--	--	--
Malic acid	--	--	--	--
α-ketoglutaric	--	--	--	--
Unidentified	--	--	--	--
Alanine	--	--	--	--
Aspartic	--	--	--	--
Glutamic	--	--	--	--
Glycine	--	--	--	--
Serine	--	--	--	--
Unidentified	--	--	--	176

Table 8. cont'd.

Fraction	Ether			80% Ethanol
	Neutral counts, dpm[b]	Acidic counts, dpm[b]	Alkaline counts, dpm[b]	
Glucose	--	--	--	11
Sucrose	--	--	--	384
Unidentified	--	--	--	467
Insoluble material[d]				
Ether insoluble 174	--	--	--	--
Ethanol insoluble	--	--	--	238
Remained in sand[e]	646			

[a] Initial added to plant in moist sand.

[b] 38% pf the carrots died over the growing season.

[c] Not a sequential extraction.

[d] Sand extracted with hexane and methanol.

Distribution of label from 3-chloroallyl alcohol-^{14}C-U

Ether extracts. The distribution of label from 3-chloroallyl alcohol in plant products is shown in Table 9. The neutral ether fraction from bush beans, tomatoes and carrots contained label in carotenes, other ether soluble pigments, lipids and unidentified compounds. The acidic ether fraction contained labelled pigments, lipids, anionic acids and unidentified compounds. The alkaline ether extract contained label in pigments, lipids, unidentified compounds and cationic acids.

80% ethanol extract. The 80% ethanol extract from bush beans, tomatoes and carrots was divided into anionic acids, cationic acids and neutral compounds as sumarized in Table 10. Label in the anionic acid fraction was distributed between citric acid, succinic acid, fumaric acid, malic acid and α-ketoglutaric acid. There were several unidentified anionic acids in this fraction. Cationic acids such as alanine, aspartatic, glutamic, serine, glycine and unidentified acids were found in the cationic fraction. Sugars such as glucose and sucrose were found in the neutral fraction as well as some unidentified compounds.

Label in carrot after 1 growing season. The distribution of label from 3-CAA-^{14}C-U in ether fractions, 80% ethanol fractions, insoluble fraction and sand is shown in Table 11. The majority of the label in the ether soluble fraction appeared in pigments, anionic acids, cationic acids and unidentified compounds. The 80% ethanol fraction contained labelled citric acid in the anionic fraction, labelled serine in cationic fraction and glucose and sucrose in the neutral fraction. The insoluble ether and ethanol

residues contained label but the compounds were not identified. The sand growing support contained label that was not identified.

Absorption with time. The absorption of 3-chloroallyl alcohol from a nutrient solution is shown in Figure 4. The maximum absorption occurred between 18 and 24 hours and leveled off. The 3-CAA was absorbed from 60 to 75% of the total label given to the plant in 24 hr.

Short term residue analysis

Dichloropropenes

Bush beans. Bush beans demonstrated the ability to absorb 1,3-DCPE rapidly from vermiculite growth medium (Table 13). Residues of cis- and trans-1,3-DCPE appeared in the plant 0.5 hr after introduction in the vermiculite/plant system. The maximum concentration in the plant was at 4 hr after inoculation. By 24 hr, levels of 1,3-DCPE were reduced to less than ng/gm fresh weight on a tissue basis.

Appearing approximately 2 hr after inoculation of 1,3-DCPE to the plant were traces of 3-CAA. Levels of cis- and trans- 3-CAA reached maximum at 6 to 10 hr and decreased rapidly so that no residue was detected by 48 hr.

Tomatoes. Tomato plants absorbed cis- and trans-1,3-DCPE from the vermiculite medium as shown in Table 14. Maximum levels of 1,3-DCPE were detected from 2 to 4 hr and decreased beyond detection by 48 hr.

Carrots. Carrots absorbed cis- and trans-1,3-DCPE readily as shown in Table 15. Levels of cis-1,3-DCPE increased to 13 ng/gm by 2 to 4 hrs and decreased to the limits of detection by 16 hr.

The trans-1,3-DCPE reached maximum concentration of 8 ng/gm at 2 to 4 hr and slowly decreased to 0.7 ng/gm by 48 hr.

Residues of 3-CAA were identified in the carrot beginning at 2 hr after inoculation of the carrot with the parent 1,3-DCPE. The alcohol reached maximum levels at 6 to 10 hrs and decreased to 0.5 to 1.25 ng/gm by 48 hr.

3-Chloroallyl alcohol

Bush beans. Bush beans absorbed cis- and trans-3-CAA from a vermiculite medium. Residues were detected at 1 hr, reached a maximum at 4 hr and decreased beyond detection by 48 to 72 hr (Table 16). Associated with the 3-CAA treatment of bush beans was advanced stages of phytotoxicity as evidenced by grey-black mottled areas appearing on the leaves and apparent loss of turgor.

The 3-chloroallyl alcohol was transformed in the plant to 3-chloroacrylic acid and 3-chloro-1-propanol. These two compounds reached maximum concentrations 8 to 16 hr after administration of the parent 3-CAA. The trans- isomer of 3-chloroacrylic acid was identified by retention time in the GLC while the cis- isomer was not identified by retention time as there was no standard available for comparison.

Tomatoes. Tomatoes absorbed 3-CAA from a vermiculite medium as shown in Table 17. Maximum concentrations were observed at 4 to 6 hrs and subsequently, they decreased below detection by 48 hr.

The metabolites trans-3-chloroacrylic acid and 3-chloro-1-propanol appeared maximal at 8 to 16 hr after inoculation with

3-CAA. The 3-chloroacrylic acid was not detectable after 24 hr while the 3-chloro-1-propanol remained detectable for up to 48 hr after inoculation.

Carrots. Carrots absorbed and transformed cis- and trans-3-CAA as shown in Table 18. Maximum levels of 3-CAA were observed 2 to 4 hr after inoculation and decreased below detectable levels by 48 hr. Material was located in both the root and shoot of the carrot.

The metabolites trans-3-chloroacrylic acid and 3-chloro-1-propanol reached maximal levels in the carrot at 8 to 16 hr after inoculation with 3-CAA. They decreased in concentration until 96 hr when no detectable residues remained.

Partition coefficients

The partition coefficients of 1,3-dichloropropene and 3-chloroallyl alcohol between n-octanol and water are given in Table 12. The 1,3-DCPE has a p-value of 54-08 while the 3-CAA has a p-value of 3.8 at 60 C and pH 6.8.

Identification of dichloropropenes and metabolites

The dichloropropenes were identified by two methods. The first method employed GLC and comparison of retention times of standard compounds and samples from the plant (cis- isomer: 216 seconds; trans- isomer: 252 seconds). The second method of identification was mass spectrometry of standard and samples from the plant. The mass spectra is definitive and the major ion peaks are shown in Figure 5.

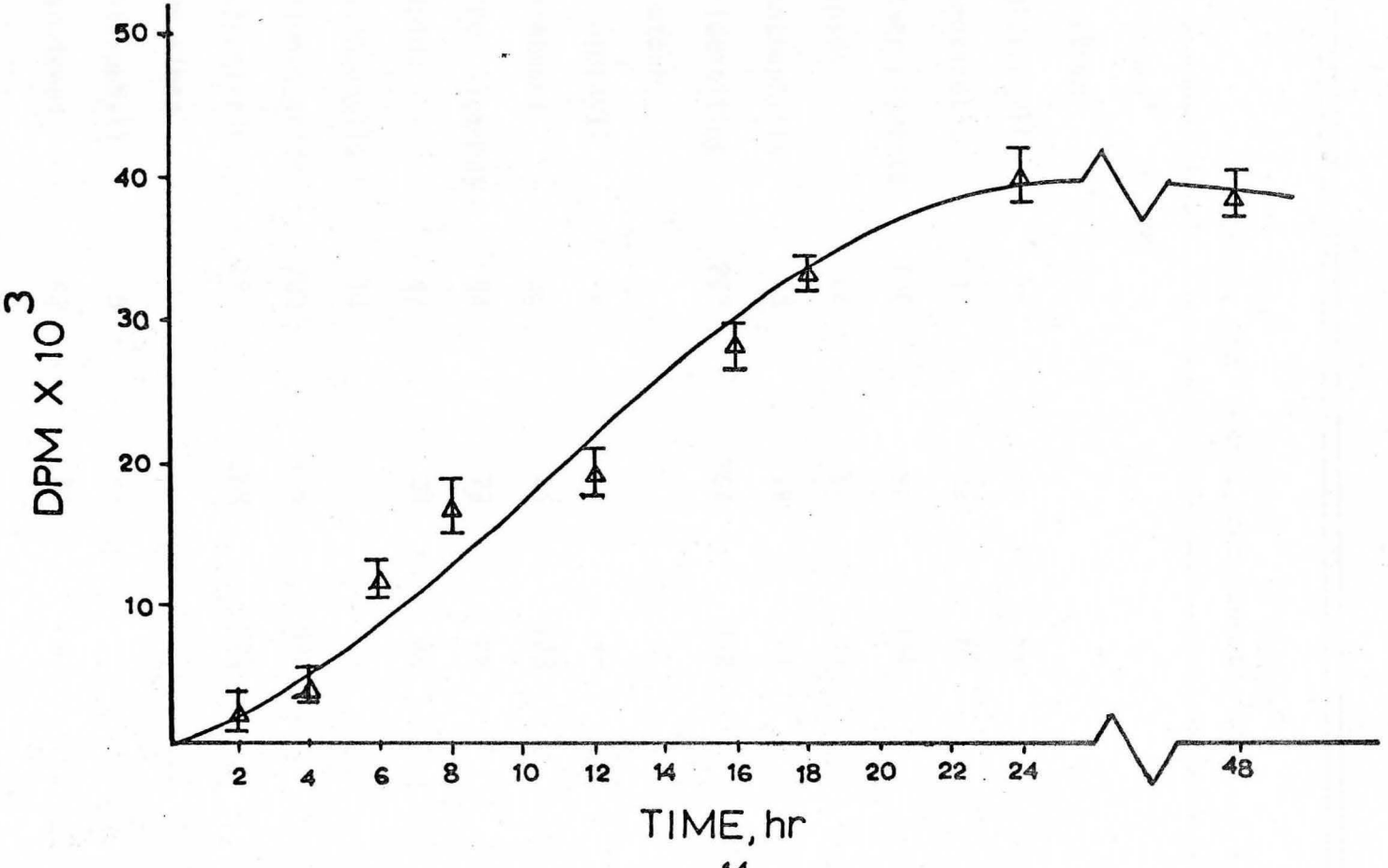

Figure 4. Absorption of 3-chloroallyl alcohol-^{14}C-U in bush beans

Table 9. Distribution of label from 3-chloroallyl alcohol-^{14}C-U in neutral ether (pH 7), acidic ether (pH 1) and alkaline ether (pH 13) in bush bean, tomato and carrot after 24 hr.

Fraction	Bean[b] counts, dpm	Tomato[b] counts, dpm	Carrot[b] counts, dpm
Initial added[a] 26,500			
Neutral ether			
Chlorophyll	--	--	--
Carotenes	41	32	67
Other pigments	106	96	134
Lipids	66	52	84
Xanthophylls	13	18	21
Unidentified	243	264	462
Acidic ether			
Chlorophyll	--	--	--
Carotenes	36	45	173
Other pigments	84	73	65
Lipids	47	38	82
Xanthophylls	10	--	--
Anionic acids	783	935	892
Unidentified	421	368	511
Alkaline ether			
Chlorophyll	5	--	--
Carotenes	52	40	97

Table 9. Cont'd.

Fraction	Bean[b] counts, dpm	Tomato[b] counts, dpm	Carrot[b] counts, dpm
Alkaline ether			
Other pigments	75	113	97
Lipids	46	52	108
Saponifiable lipids	31	30	47
Cationic acids	924	1034	742
Unidentified	684	597	682

[a] Initial added to plant nutrient solution.

[b] Average values of 4 replications.

Table 10. Distribution of label from 3-chloroallyl alcohol-^{14}C-U in 80% ethanol extract from bush bean, tomato and carrot after 24 hr.

Fraction	Bean[b] counts, dpm	Tomato[b] counts, dpm	Carrot[b] counts, dpm
Initial added[a] 26,500			
Anionic acids			
Citric	182	172	183
succinic	136	114	146
Fumaric	83	102	86
Malic	108	93	74
∝-ketoglutaric	65	46	57
Unidentified	347	474	392
Cationic acids			
Alanine	43	37	46
Aspartic	26	12	22
Glutamic	52	68	43
Serine	13	18	--
Glycine	64	43	17
Neutral Compounds			
Glucose	435	341	298
Sucrose	966	810	1065

Table 10. Cont'd.

Fraction	Bean[b] counts, dpm	Tomato[b] counts, dpm	Carrot[b] counts, dpm
Neutral compounds			
Unidentified	608	496	308

[a] Initial added to plant in nutrient solution.

[b] Average values of 4 replications.

Table 11. Distribution of label from 3-chloroallyl alcohol-^{14}C-U in carrot after 1 growing season (6 months).

Fraction	Ether[c]			80% Ethanol
	Neutral counts, dpm[b]	Acidic counts, dpm[b]	Alkaline counts, dpm[b]	counts, dpm[b]
Initial added[a]	61,500			
Chlorophyll	--	--	--	--
Carotenes	81	73	56	--
β-carotene	11	--	--	--
Other pigments	8	24	31	--
Lipids	43	61	51	
Saponifiable Lipids	--	--	38	--
Xanthophylls	--	--	--	--
Anionic acids	--	107	--	--
Cationic acids	--	--	82	--
Unidentified	108	197	173	--
Anionic acids				
Citric	--	--	--	12
Succinic	--	--	--	--
Fumaric	--	--	--	--
Malic	--	--	--	--
α-ketoglutaric	--	--	--	--
Unidentified	--	--	--	109

Table 11. cont'd.

Fraction	Ether[c]			80% Ethanol
	Neutral counts, dpm[b]	Acidic counts, dpm[b]	Alkaline counts, dpm[b]	counts, dpm[b]
Cationic acids				
Alanine	--	--	--	--
Aspartate	--	--	--	--
Glutamic	--	--	--	--
Glycine	--	--	--	--
Serine	--	--	--	10
Unidentified	--	--	--	--
Neutral compounds				
Glucose	--	--	--	23
Sucrose	--	--	--	186
Unidentified	--	--	--	226
Insoluble material[d]				
Ether	132	--	--	--
Ethanol	--	--	--	187
Remained in sand[e]				
Unidentified	422	--	--	--

[a] Initial added to plant in moist sand

[b] 51% of the carrots died over the growing season

[c] Not a sequential extraction

[d] Not digested or identified

[e] Sand extracted with hexane and methanol

Table 12. Partition coefficient of 1,3-dichloropropene and 3-chloroallyl alcohol in an octanol/water system.

Compound	Counts, dpm	p-value
1,3-dichloropropene-^{14}C-U		
Octanol	108,228 ± 5414	
Water	2,001 ± 429	
		54.08[a]
3-chloroallyl alcohol-^{14}C-U		
Octanol	276,661 ± 15,582	
Water	71,364 ± 2,566	
		3.876

[a] p-value = [Compound in octanol]/[Compound in water phase].

Table 13. Dichloropropene residues and immediate metabolites in bush beans.

Time, hr	Compound (ng/gm)[a]				
	c-1,3-DCPE	t-1,3-DCPE	c-3-CAA[b]	t-3-CAA[b]	t-3-CAcryl[c]
0	---	---	---	---	---
0.5	2.68±0.52	1.4±0.40	---	---	---
1	3.65±0.70	3.06±0.25	---	---	---
2	5.34±1.1	3.80±0.34	0.58±0.26	0.51±0.16	---
4	1.50±0.15	1.62±0.06	1.87±0.40	2.84±0.47	---
8	0.87±0.08	0.60±0.12	2.36±0.74	3.20±0.53	---
16	0.10±0.02	0.34±0.08	1.15±0.37	1.73±0.46	---
24	0.08±0.00	0.18±0.05	0.12±0.05	0.46±0.16	---
48	---	---	---	---	---
72	---	---	---	---	---
96	---	---	---	---	---
120	---	---	---	---	---

[a] Expressed as ng/gm on wet weight basis.

[b] 3-CAA as the TFA derivative.

[c] 3-CAcryl as the TMS derivative.

Table 14. Dichloropropene residues and immediate metabolites in Tomatoes.

Time, hr	Compound (ng/gm)[a]				
	c-1,3-DCPE	t-1,3-DCPE	c-3CAA[b]	t-3-CAA[b]	t-3-CAcryl[c]
0	---	---	---	---	---
0.5	---	---	---	---	---
1	1.20±0.11	0.35±0.06	---	---	---
2	1.42±0.56	0.90±0.20	---	---	---
4	1.50±0.30	0.55±0.24	0.84±0.30	0.17±0.02	---
8	0.27±0.0	0.16±0.20	0.73±0.40	0.14±0.06	---
16	0.07±0.01	0.04±0.02	0.24±0.08	0.10±0.06	---
24	0.02±0.00	0.01±0.00	---	---	---
48	---	---	---	---	---
72	---	---	---	---	---
96	---	---	---	---	---
120	---	---	---	---	---

[a]Expressed as ng/gm on wet weight basis.

[b]3-CAA as the TFA derivative.

[c]3-CAcryl as the TMS derivative

Table 15. Dichloropropene residues and immediate metabolites in Carrots

Time, hr	Compound (ng/gm)[a]				
	c-1,3-DCPE	t-1,3-DCPE	c-3-CAA[b]	t-3-CAA[b]	t-3-CAcryl[c]
0	---	---	---	---	---
0.5	---	---	---	---	---
1	2.95±0.24	2.65±0.10	---	---	---
2	13.60±1.5	8.20±1.32	2.84±0.46	1.75±0.80	---
4	6.56±1.40	4.35±1.51	6.46±2.18	5.86±1.62	---
8	1.19±0.24	3.46±1.96	8.16±1.32	8.90±2.30	---
16	---	1.40±0.10	3.85±0.54	5.15±1.80	---
24	---	0.85±0.04	2.46±0.80	4.05±1.20	---
48	---	0.70±0.05	0.51±0.20	1.25±0.04	---
72	---	---	---	---	---
96	---	---	---	---	---

[a] Expressed as ng/gm on wet weight basis.

[b] 3-CAA as the TFA derivative.

[c] 3-CAcryl as the TMS derivative.

Table 16. 3-Chloroallyl alcohol residues and immediate metabolites in Bush Beans.

Time, hr	Compound(ng/gm)[a]			
	c-3-CAA[b]	t-3-CAA[b]	t-3-CAcryl[c]	3-C-1-P[d]
0	---	---	---	---
0.5	---	---	---	---
1	8.3±1.6	6.2±1.9	---	---
2	14.1±2.7	11.6±3.4	1.84±0.7	---
4	32.4±3.8	27.4±2.6	6.41±1.1	4.5±1.3
8	13.0±2.7	11.2±3.1	8.5±2.4	7.1±2.1
16	2.6±0.82	6.36±1.2	7.2±2.1	3.4±1.2
24	0.18±0.10	3.10±0.74	4.1±1.0	1.40±0.8
48	---	1.3±0.26	1.8+0.4	0.8±0.2
72	---	---	---	---
96	---	---	---	---

a Expressed as ng/gm on wet weight basis.

b 3-CAA as the TFA derivative.

c 3-CAcryl as the TMS derivative.

d 3-C-1-P as the TFA derivative of 3-chloro-1-propanol.

Table 17. 3-Chloroallyl alcohol residues and immediate metabolites in Tomatoes.

Time, hr	Compound(ng/gm)[a]			
	c-3-CAA[b]	t-3-CAA[b]	t-3-CAcryl[c]	3-C-1-P[d]
0	---	---	---	---
0.5	---	---	---	---
1	16.4±1.6	13.7±2.6	---	---
2	24.6±2.3	22.1±3.5	1.2±0.34	--
4	41.2±4.5	37.4±3.2	6.5±0.92	1.6±0.24
8	11.2±3.2	18.2±2.7	8.2±1.34	2.3±0.41
16	2.5±0.82	4.2±1.2	5.3±1.83	2.6±1.20
24	0.8±0.08	1.4±0.24	1.6±0.64	1.9±0.73
48	---	---	---	0.4±0.18
72	---	---	---	---
96	---	---	---	---

[a] Expressed as ng/gm on wet weight basis

[b] 3-CAA as the TFA derivative.

[c] 3-CAcryl as the TMS derivative.

[d] 3-C-1-P as the TFA derivative of 3-chloro-1-propanol.

Table 18. 3-Chloroallyl alcohol residues and immediate metabolites in Carrots.

Time, hr	Compound(ng/gm)[a] c-3-CAA[b]	t-3-CAA[b]	t-3-CAcryl[c]	3-C-1-P[d]
0	---	---	---	---
0.5	---	---	---	---
1	16.1±1.8	13.2±2.1	2.6±0.7	---
2	32.1±3.6	42.5±3.8	9.3±1.2	1.8±0.2
4	25.2±2.2	27.3±1.9	12.4±1.0	6.4±1.1
8	11.2±2.4	14.1±1.2	13.3±2.2	4.7±0.4
16	3.6±0.73	7.5±0.81	7.2±1.0	2.5±0.36
24	0.6±0.2	5.0±0.72	3.4±0.7	2.1±0.56
48	---	2.3±0.92	1.6±0.4	1.2±0.62
72	---	---	---	0.62±0.40
96	---	---	---	---

[a] Expressed as ng/gm on wet weight basis.
[b] 3-CAA as the TFA derivative.
[c] 3-CAcryl as the TMS derivative.
[d] 3-C-1-P as the TFA derivative of 3-chloro-1-propanol.

The 3-chloroallyl alcohol was identified by GLC and mass spectrometry. In order to volatilize and attain a sharp peak on the GLC, a TFA derivative had to be formed of the 3-CAA. Retention times could then be compared with standards and plant samples (cis-isomer: 148 seconds; trans- isomer: 163 seconds). The mass spectra of 3-CAA-TFA standard and 3-CAA-TFA from plant are shown in Figure 6.

The presence of 3-chloro-1-propanol was suspected from GLC data as there was a peak of the TFA derivative from the plant material after the retention time of 3-CAA-TFA (retention time of 284 seconds). The existance was confirmed by mass spectrometry as shown in Figure 7.

The existance of 3-chloroacrylic acid in the plant was confirmed by GLC. Standard samples were compared with plant samples with respect to retention times (trans-3-chloroacrylic acid 248 seconds). Only the trans- isomer was identified as the only existing standard available was in the trans- form. The extremely low concentrations of 3-chloroacrylic acid prevented mass spectral identification of the compound.

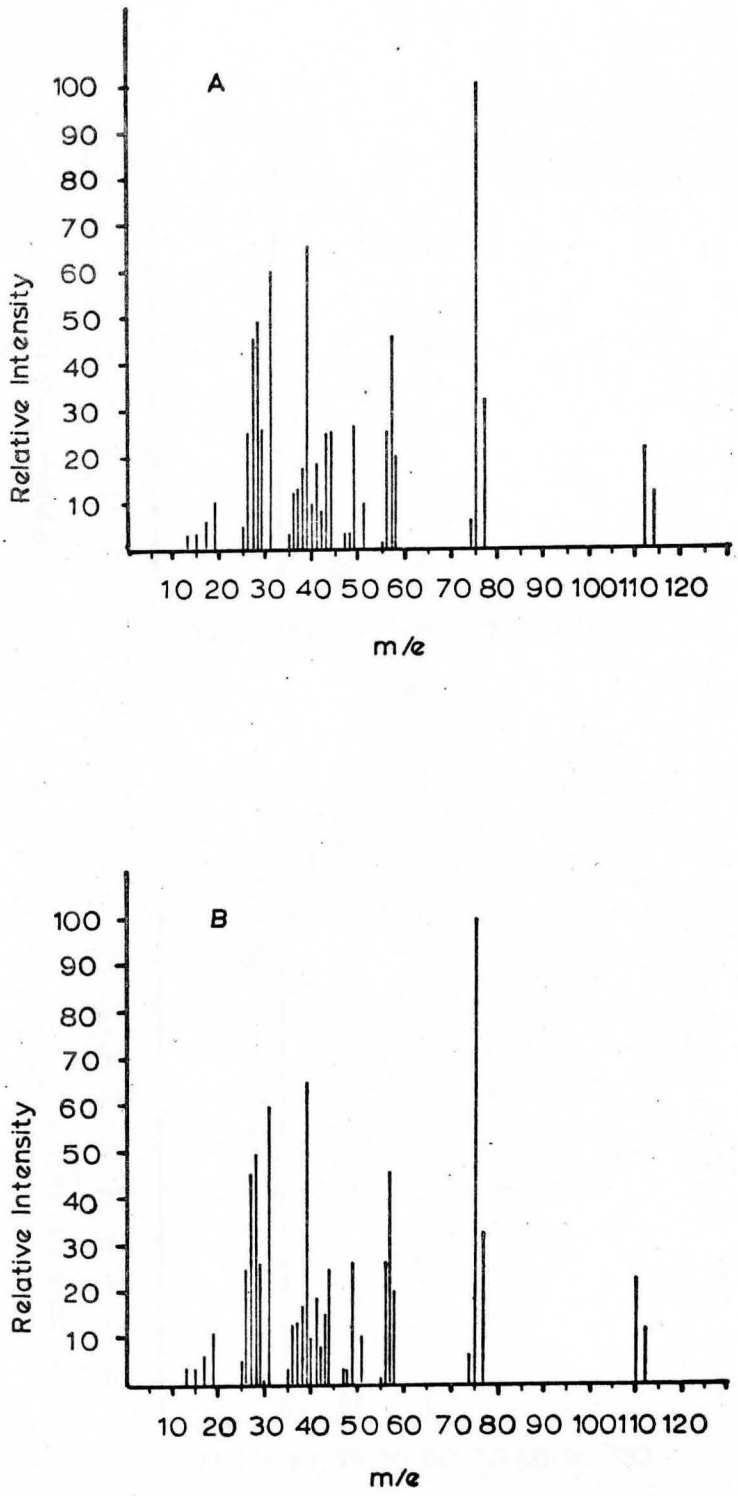

Figure 5. Mass spectra of 1,3-dichloropropene A - bean, 4 hr
B - standard

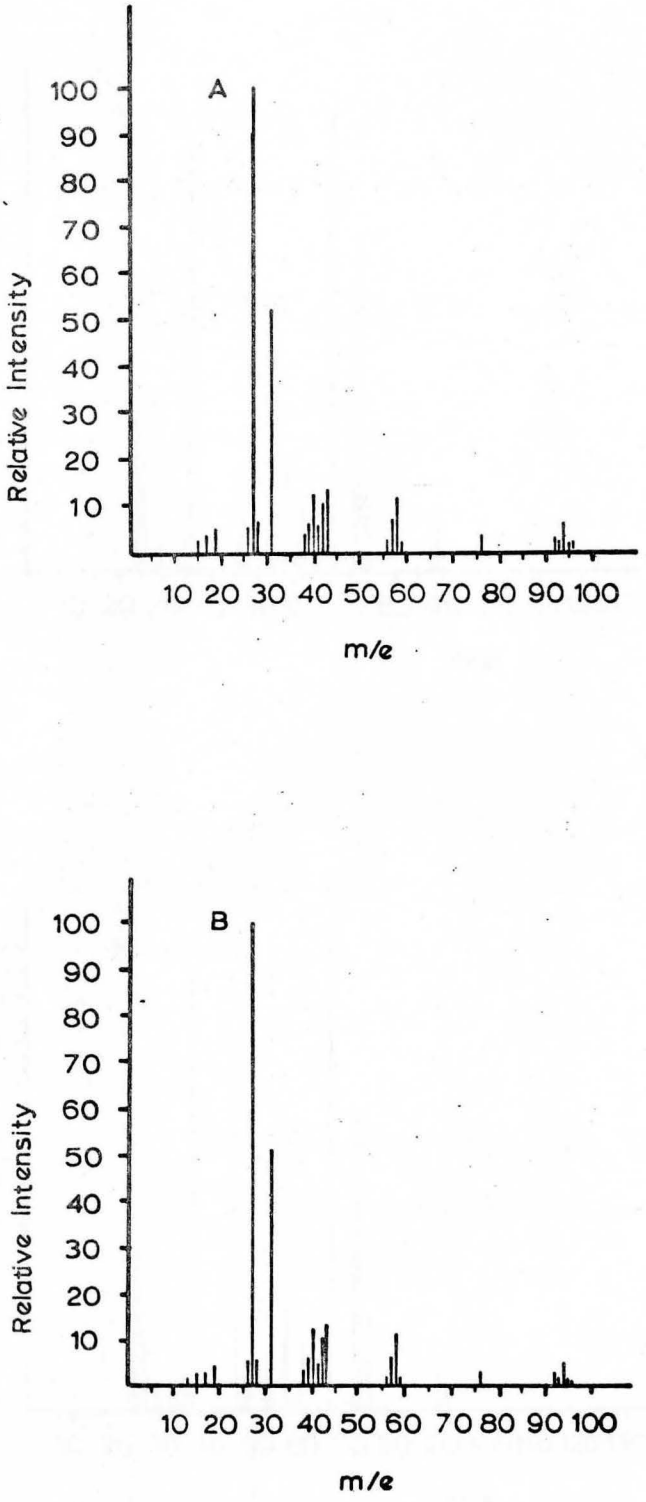

Figure 6. Mass spectra of 3-chloro-1-propanol A-bean, 8 hr
B - standard

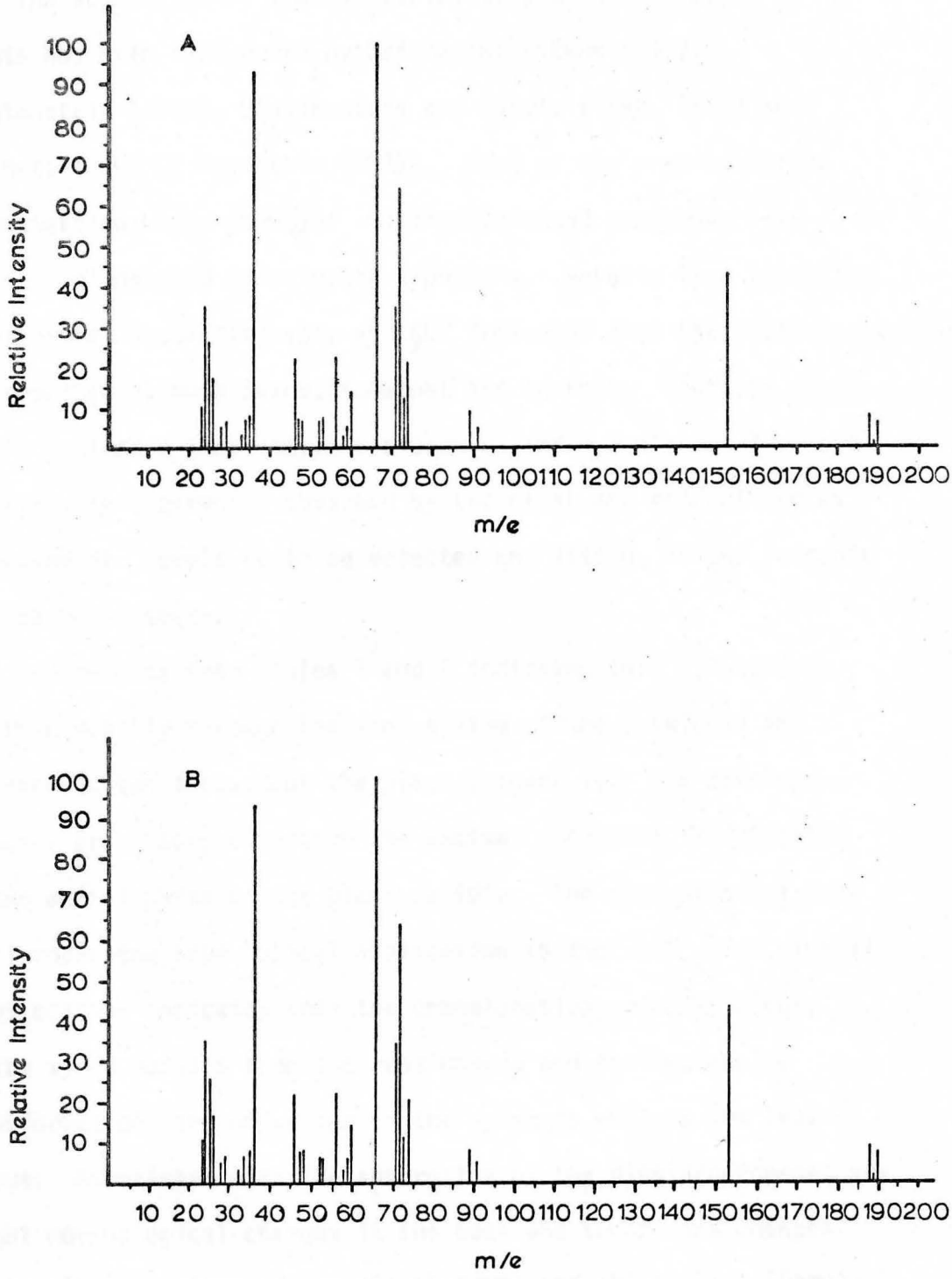

Figure 7. Mass spectra of 3-CAA-TFA. A-bean, 8 hr B-standard

DISCUSSION

The absorption of organochlorine compounds from soils by plants has been documented by Casida and Lykken (1969), Lichtenstein (1959), Lichtenstein and Schulz (1960, 1965) and Charnetski and Lichtenstein (1973). Much of the organochlorine investigations have employed multichlorinated compounds like the cyclodienes and chlorinated biphenyls. Results from labelling experiments, autoradiography and GLC indicated that the dichloropropenes are absorbed by bush beans, tomatoes and carrots. Thomason et al. (1971) indicated that ethylene dibromide and 1,2-dibromo-3-chloropropane were apparently absorbed by the plant and metabolized as increased Br^- levels could be detected and little, if any, organic bromide was detected.

The results from Tables 1 and 2 indicated that 1,3-DCPE is absorbed readily through the root system of the bush bean and is translocated throughout the plant (Figure 1). The dichloropropenes are absorbed through the excised shoot and translocated to the aerial parts of the plant rapidly. The absorption of 1,3-dichloropropene from topical application to the leaf with minimal translocation indicates that the translocation probably occurs in the xylem vessels from the root upward and that metabolic transformations are occurring in the xylem as well as the leaf tissue. Associated with the adsorption of the dichloropropenes are slight morphological changes in the bean and tomato and changes in the ultrastructure of the mitochondria and chloroplast (Campbell et al., unpublished).

The results of residue studies using bush beans, tomatoes and carrots indicate that the dichloropropenes are rapidly absorbed by the plant (Table 13, 14, 15). The GLC data confirms that the dichloropropenes are entering the plant as the dichloropropene and it also indicates that 1,3-DCPE does not remain in the plant after 48 hr. Appearing shortly after the dichloropropenes in the plant are residues that were identified as 3-chloroallyl alcohol. Residues of 3-CAA, as with the dichloropropenes, are short lived in the plant. The half life of the dichloropropenes and 3-chloroallyl alcohol in the plant are $T_{1/2}$= 1.48 hr and $T_{1/2}$= 4.36 hr respectively, which would indicate that they will not create harmful long-term residue problems in plant tissue.

Only slight differences in concentrations of the cis- and trans- isomers were detected in the plant. These differences were not statistically significant but it appeared trendwise, that the cis- isomer was reduced faster in concentration than the trans- isomer. It was apparent in phytotoxicity that the cis- isomer of 3-CAA was much more damaging to the plant than the trans- isomer.

Concurrently, bush beans, tomatoes and carrots absorbed 3-chloroallyl alcohol from the growing media. The plants absorbed higher levels of 3-CAA than the dichloropropenes and the residues were detectable for a longer duration. The 3-CAA was absorbed and translocated to the aerial portions of the plant readily and morphological damage, much greater than with the dichloropropenes, occurred by 8 hr after treatment. Ultrastructural damage to the plastids and mitochondria were noted by 4 hr after treatment

(Campbell et al., 1972). Coupled with the residue data presented in Tables 16, 17 and 18, it is apparent that the 3-chloroallyl alcohol is readily absorbed and metabolized by the plant system.

The detection of 3-chloroacrylic acid and 3-chloro-1-propanol were further evidence of absorption and metabolism of the dichloropropenes in the plant. The 3-chloroacrylic acid has been reported by Belser and Castro (1971) and Moje et al. (1957) as a bacterial metabolite of 1,3-DCPE and is presumbed to be the result of oxidation of the alcohol to an acid by an enzyme system. The 3-chloro-1-propanol probably results from the direct reduction of the allyl chloride bond to the saturated chlorinated alcohol. Once that is completed the alcohol is probably oxidized to the acid and metabolized in a similar scheme as described by Goldman et al. (1968) and Ruddick (1972) for halogen cleavage and metabolism of alcohols in bacteria and mammals eventually ending up in glycolysis as pyruvate. The 3-chloroacrylic acid has been previously described by Kearney et al. (1964) in the plant system in that it goes to a β-chloro-β-hydroxypropionate and subsequent loss of chlorine to yield pyruvate and HCl. Thus, it appears that the plant can absorb and metabolize the dichloropropenes to a central metabolite, such as pyruvate, and incorporate it directly into normal plant products. The residue levels of the dichloropropenes and the 3 metabolites are shown in Figure 8.

The longer term metabolism of the dichloropropene and 3-chloroallyl alcohol was evidenced by the incorporation of label from dichloropropenes and 3-chloroallyl alcohol into normal plant

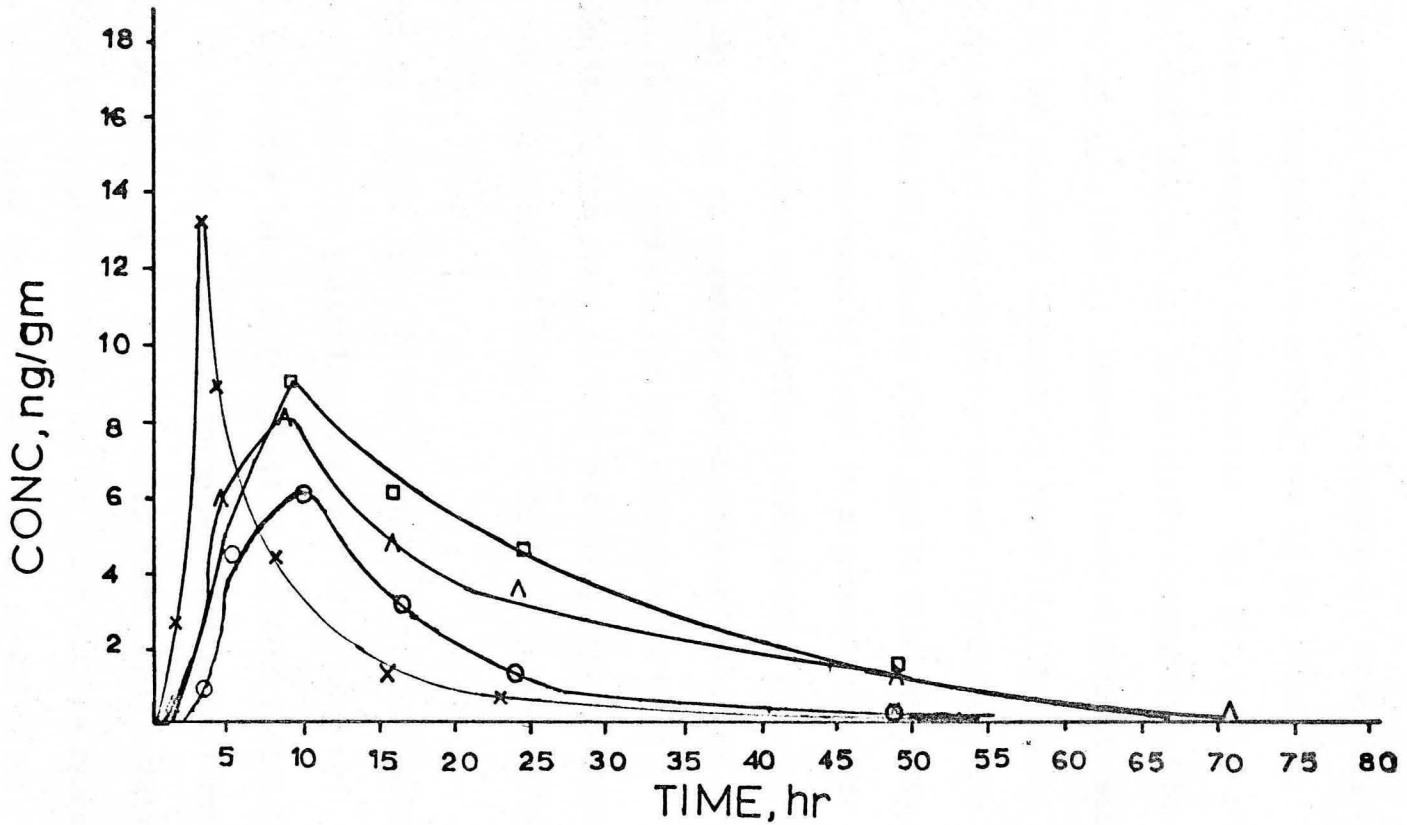

Figure 8. Residues of dichloropropenes and metabolites with time. x—x DCPE, Λ——Λ 3-CAA, O——O 3-C-1-P, □——□ 3-CAcryl

products (Tables 3 - 11). The majority of the label from both the alcohol and the dichloropropene was located in the 80% ethanol fraction. This fraction contained anionic acids, cationic acids and neutral compounds such as sugars and pigments. This data would indicate that the compounds are metabolized by the plant to a potentially central metabolic intermediate. The possibility of pyruvate being that intermediate is partially substantiated by the work of Kearney et al. (1964). However, labelled pyruvate was not isolated in the products containning label in either the anionic acid fraction of the neutral fraction. Pyruvate has a high turnover rate in a rapidly growing plant and in this case, may not be detectable. The occurrence of label in pigments and lipids in ether soluble fractions was further evidence that the majority of the label was going to a central metabolite in the plant system. Still further evidence for the incorporation into a central metabolite in the plant is the relatively high incorporation rates for 1,3-dichloropropene-^{14}C-U (55 - 80%) and 3-chloroallyl alcohol-^{14}C-U (60 - 75%).

An effort to account for all 100% of the label in the metabolic studies was not physically possible since facilities were not available to administer label to the plant in a closed photosynthetic type chamber and trap CO_2 and dichloropropenes that volatilized. It would expected that some of the dichloropropene would volatilize and escape from the solution culture and it would also be expected to have some of the label lost as ^{14}C-CO_2 due to normal plant respiration. The hexane extracts from the solution culture

remaining after incubation usually brought the total accountable radioactivity up to about 80% of that administered initially.

The role of conjugation in the plant metabolism of the dichloropropenes is not known nor was it specifically determined. There was label ramaining in insoluble fractions of ether and ethanol extracts and some of this label may have been in the form of conjugates. Additionally, some of the compounds not identified in the ethanol neutral fraction could have been conjugated with sugars and glycosides. Other potential conjugates would be expected with the amino acids and perhaps associated with lipid phase of the ether extraction.

The potential metabolism of the dichloropropenes in the plant is given in Figure 9. The elimination of chlorine to give an alcohol (3-CAA) has been described by Belser and Castro (1971). The subsequent oxidation of the alcohol to 3-chloroacrylic acid and the reduction of the allyl chloride bond to yield 3-chloro-1-propanol has not been described in association with the plant metabolism of these compounds previous to this time. Subsequent metabolism of 3-chloroacrylic acid and 3-chloro-1-propanol was not possible but from evidence of labelling patterns, it is highly probable that a central metabolite,such as pyruvate, results from the dichloropropenes and is subsequently incorporated into normal plant metabolism.

The long term metabolism of the dichloropropenes in the carrot indicated that the carrot absorbs and metabolizes the dichloropropenes and 3-chloroallyl alcohol. The label located in the plant was much

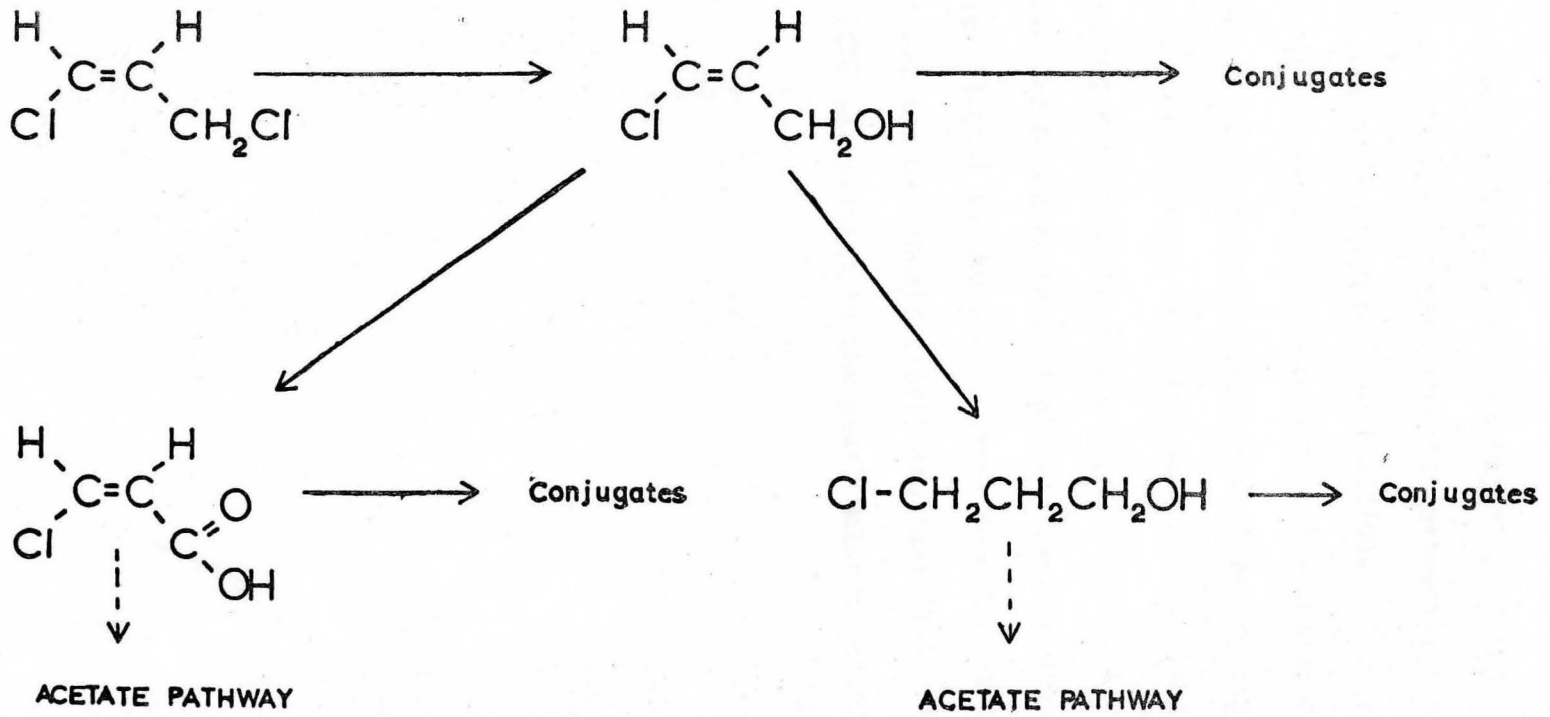

Figure 9. Plant metabolism of 1,3-dichloropropene

lower than the short term metabolic studies and it was distributed in different areas of the plant. The majority of the label from both sources was located in the pigments (primarily carotenes), unidentified compounds and insoluble fractions. The lack of label in the TCA acids and other metabolically active compounds indicate that the compound was fully metabolized by the plant and perhaps the majority of the label absorbed by the plant was given off during respiration. GLC analysis of long term plant material revealed no detectable levels of 1,3-dichloropropene or 3-chloroallyl alcohol in the plant. These results coincided with those of Karasz and Gantenbein (1971) and Tams (1945) that no detectable DCPE was evident in the plant material after 1 growing season.

SUMMARY AND CONCLUSIONS

The absorption, translocation and metabolism of 1,3-dichloropropene in bush beans, tomatoes and carrots was investigated. The absorption of dichloropropene was monitored through the use of GLC and 1,3-dichloropropene-^{14}C-U. Translocation and metabolism by the plant was monitored using 1,3-dichloropropene-^{14}C-U ; GLC and GC-MS. The absorption, translocation and metabolism of 3-chloroallyl alcohol was investigated in bush beans, tomatoes and carrots employing techniques used for the dichloropropenes.

Bush beans, tomatoes and carrots absorbed the dichloropropenes rapidly. By 0.5 hr after treatment, dichloropropenes were found in the plant and by 40 to 6 hr maximal levels were obtained. The dichloropropene residues decreased below detectable levels by 48 hr. The 3-chloroallyl alcohol was absorbed rapidly by the plant and reached maximal levels 8 to 12 hr after treatment. 3-Chloroallyl alcohol decreased in concentration to below detectable levels by 72 hr. The incorporation studies indicated that maximal uptake of 1,3-dichloropropene-^{14}C-U and 3-chloroallyl alcohol-^{14}C-U occurred by 24 to 36 hr.

Short term metabolic studies with 1,3-dichloropropene and 3-chloroallyl alcohol indicated that the dichloropropene is converted to 3-chloroallyl alcohol and subsequently to 3-chloroacrylic acid and 3-chloro-1-propanol. These compounds were short lived in the plant and were not detectable after 72 hr from treatment time with the dichloropropenes. The metabolites were identified by GLC and GC-MS.

Longer term metabolic studies with 1,3-dichloropropene-^{14}C-U and 3-chloroallyl alcohol-^{14}C-U indicated that the label was located in normal plant products such as metabolic acids, amino acids, sugars, lipids and unidentified compounds. The label distribution indicated metabolism via acetate pathway (perhaps as a pyruvate intermediate). The appearance of label in lipids and pigments was not high and was characteristic of a young rapidly metabolizing plant. Label pattern was similar to that expected by feeding the plant pyruvate or acetate.

Long term (6 month) studies with carrots indicated that label from 1,3-dichloropropene and 3-chloroallyl alcohol was located in metabolic acids, sugars and insoluble material in the plant. There was some unidentified labelled material remaining in the sand after the growing season.

The role of conjugation as a means of metabolic elimination of the dichloropropenes and 3-chloroallyl alcohol was not fully explored. It is expected that the alcohol, 3-chloroacrylic acid and 3-chloro-1-propanol would all form conjugates with sugars, amino acids and glycosides.

The dichloropropene compounds that are absorbed by the plant and metabolized by the plant, culminate as normal plant products. Hence, they are not potential residue problems in the plant and environmental concern about plant residues should be minimal.

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